# Significance of fat cell size on liver and visceral fat accumulation in metabolic syndrome; impact of dietary fructose

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Tiivistelmä – Referat – Abstract

*Bakgrund:* Det metabola syndromet predisponerar för kardiovaskulära sjukdomar och karaktäriseras av bukfetma, insulinresistens, hypertension samt protrombotiska och proinflammatoriska tillstånd. Alla obesa människor utvecklar trots allt inte metabolt syndrom. Hypertrofi av fettcellerna i underhuden har förknippats med metabola komplikationer vid obesitet. Syftet med undersökningen var att bedöma om högt fruktosintag påverkar storleken på fettcellerna i underhuden och om fettcellernas storlek kan vara en avgörande metabol riskfaktor hos manliga personer med obesitet och metabolt syndrom.

*Metoder:* För att bedöma fruktosens metabola effekter konsumerade 34 manliga obesa personer dagligen drycker sötade med fruktos, totalt 75 g tillsatt fruktos/dag, i 12 veckors tid. Biopsier från fettvävnaden i underhuden erhölls genom nålaspiration och fettcellernas storlek mättes under ett ljusmikroskop. Även mängden leverfett och övriga kardiovaskulära och metabola riskparametrar analyserades.

*Resultat:* Tillsatt fruktos i dieten under 12 veckors tid påverkade inte storleken på fettcellerna i underhuden hos personer med obesitet (P=0,417). Försökspersonerna hade högre triglyceridvärden (P=0,034) och ökad mängd leverfett (P=0,011) efter fruktosinterventionen. Undergrupper av försökspersoner med stora och små fettceller (under eller över medianvärdet) svarade olika på det ökade fruktosintaget beträffande ökning i mängden leverfett.

*Slutsats:* Storleken på fettcellerna i underhuden var inte en betydande faktor för den metabola hälsan hos män med obesitet och metabolt syndrom. Tillsatt fruktos i kosten på 75 g/dag under 12 veckor medförde inte förändringar i storleken på fettcellerna. Försökspersoner vars fettceller minskade i storlek efter fruktosinterventionen påvisade en ökning i mängden leverfett. Oförmågan hos fettcellerna i underhuden att expandera kan vara en bidragande faktor till utvecklingen av metabolt syndrom hos dessa personer.

Avainsanat – Nyckelord – Keywords

Adipocyte size, cardiovascular risk, dietary fructose, metabolic syndrome

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| Muita tietoja – Övriga uppgifter – Additional information |

# Contents

# 1. Introduction

The metabolic syndrome is a consequence of obesity and constitutes a cluster of risk factors for developing type 2 diabetes and cardiovascular disease (1). Major characteristics of the metabolic syndrome are abdominal obesity, dyslipidemia, hypertension, insulin resistance and prothrombotic and proinflammatory states (2).

All obese people do not develop metabolic syndrome. Different patterns in adipose tissue expansion, hypertrophy or hyperplasia, have been suggested to predict metabolic complications in obesity. Hypertrophic adipocytes are associated with cardiovascular disease, insulin resistance, dyslipidemia and hepatic steatosis, and predicts the development of type 2 diabetes. (3-5)

The increase in the prevalence of obesity, type 2 diabetes and cardiovascular diseases coincides closely with the rise in dietary sugar intake. Driving the trend has particularly been the high consumption of sugar-sweetened beverages (SSBs), which in the US accounts for almost 50 % of all added sugar intake. In the US SSBs are largely sweetened with high fructose corn syrup (HFCS) which contains approximately 50 % fructose and 50 % glucose. In Europe SSBs are primarily sweetened with sucrose, which also comprises of 50 % fructose and 50 % glucose. (6)

Thus, SSBs accounts for the majority of fructose intake in the diet, either from HFCS or sucrose, and the relation between SSBs and cardiometabolic diseases seen in epidemiological studies reflect the combined effect of glucose and fructose or unique effects of fructose alone (6). Fructose particularly have been associated with increased plasma total cholesterol, postprandial triglycerides and uric acid, as well as higher systolic blood pressure, all of which are risk factors for cardiovascular disease (7-9).

The effect of high fructose consumption on SAT adipocyte size and function is unknown. Fructose consumption is, however, strongly associated with the development of nonalcoholic fatty liver disease and other markers of metabolic disease (7,10). The aim of this study is to evaluate whether added dietary fructose intake of 75 g/day for 12

weeks served as a lemonade together with ad libitum diet has an impact on subcutaneous adipose tissue cell size in males with moderate obesity. In the present study we evaluated if fat cell size is a metabolic determinant in obese males with metabolic syndrome. We also want to study if fat cell size changes during fructose consumption are associated with changes in lipid profile, fat depots or insulin sensitivity.

# 2. Review of the literature

### *2.1 Adverse metabolic effects of fructose*

Liver and adipose tissue are the most important tissues regulating metabolic homeostasis. These tissues are also vital in metabolic adverse changes elicited by fructose consumption. Fructose consumption promotes hepatic lipogenesis and suppresses mitochondrial fatty acid oxidation, thereby promoting both hepatic storage of lipids and secretion of triglyceride-rich VLDL particles (7). Hepatic steatosis leads to hepatic insulin resistance, promotion of hepatic gluconeogenesis and hyperglycemia, which contributes to the development of diabetes type 2. Hepatic secretion of triglyceride-rich VLDL particles leads to increased uptake of triglycerides in ectopic depots, leading to insulin resistance in these tissues, additionally augmenting the risk for developing diabetes type 2. (10) Increased triglyceride uptake in adipose tissue may contribute to pathological adipose tissue expansion, thereby causing low-grade chronic inflammation and aberrant secretion of adipokines (4). Fructose consumption have also been associated with increased systolic blood pressure, which may be caused by increased generation of uric acid in liver (7-9). Hypertension accompanied by dyslipidemia augments the risk for cardiovascular diseases. Furthermore, excess consumption of fructose may, due to its delivery of extra energy, lead to weight gain and fat accumulation in ectopic depots (6,11,12).

### *2.2 Fructose and NAFLD*

Nonalcoholic fatty liver disease (NAFLD) is a liver manifestation of the metabolic syndrome and the prevalence of NAFLD has paralleled that of obesity, type 2 diabetes and the metabolic syndrome (10). NAFLD is a condition of aberrant lipid accumulation

in hepatocytes and several studies have linked high fructose consumption to NAFLD (7,13-15). The liver is the primary site for fructose metabolism as it possesses GLUT5 transporters and thus is exposed to nearly 100 % of the dietary fructose load (16). Fructose is also absorbed via the portal vein leading to much higher concentrations of fructose delivered to the liver than to other tissues (10).

Intrahepatic lipid accumulation is a result of hepatic de novo lipogenesis (DNL), increased uptake of free fatty acids and/or increased dietary fat intake (17-19) . A central abnormality in NAFLD is enhanced DNL, a process by which lipids are endogenously synthesized from dietary sources or stored energy depots. Fructose metabolism in the liver directly enhances DNL by upregulating enzymes involved, and because this stimulation does not require the presence of insulin, fructose can promote hepatic lipid accumulation in the setting of insulin resistance (20-24). Fructose also bypasses the key rate limiting step in glycolysis at the level of phosphofructokinase, allowing it to act as an unlimited substrate for DNL (10). Furthermore, fructose intake has been linked to a reduction in β-hydroxybutyrate which indicates a reduced ability of fatty acid oxidation in the liver (7). Hepatic secretion of triglyceride-rich VLDL particles leads to increased uptake of triglycerides in ectopic depots consequently causing insulin resistance and inflammation in these tissues (10). Increased levels of circulating triglycerides is also a major risk factor for atherosclerosis and cardiovascular disease.

NAFLD have been associated with increased release of diabetogenic hepatokines, such as retinol binding protein (RBP)-4, fetuin-A, fibroblast growth factor (FGF)-21 and inflammatory cytokines such as C-reactive protein, TNF-alfa and IL-6. The abnormal secretion of hepatokines may lead to increased gluconeogenesis, decreased glycogen synthesis and insulin resistance in the liver thus promoting hyperglycemia and diabetes type 2. (25)

Fructose metabolism in liver also produces uric acid due to the depletion of hepatic intracellular ATP (8,9). Uric acid acts within vascular smooth muscle to inhibit endothelial nitric oxide synthase, which leads to a decreased production of nitric oxide and thus promotion of hypertension, which is a risk factor for atherosclerosis and cardiovascular disease.

### *2.3 Fructose and adipose tissue*

The main function of the subcutaneous adipose tissue (SAT) is the storage and release of fatty acids depending on nutritional state. Adipose tissue stores excess energy in the form of triglycerides (TGs) which in times of starvation are to be released as free fatty acids and glycerol and serve as energy supply for the rest of the body. The adipose tissue also functions as a large endocrine organ and releases adipokines that are important regulators of lipid and glucose metabolism, appetite, angiogenesis, blood pressure and mediators of inflammation and insulin signaling. (26)

The capacity of the SAT to expand due to a positive energy imbalance is limited and depends on the ability of the existing adipocytes to enlarge, that is hypertrophy, or the ability of recruiting new cells and the differentiation of preadipocytes, that is hyperplasia. Inability of recruiting new adipocytes during energy excess lead to inappropriate hypertrophic expansion of existing adipocytes and adipocyte stress, which in turn accounts for many adverse effects of obesity, such as dyslipidemia, alterations in adipocytokine release and low-grade inflammation. (27,28) Klöting et al. showed a correlation between adipocyte hypertrophy and dyslipidemia, inflammation and impaired glucose homeostasis in obesity. They also showed that adipocytes were smaller in obese individuals without metabolic disease compared to obese subjects with metabolic complications. (29)

Hypertrophic expansion and exceeded storage capacity of the adipose tissue lead to spillover of lipids and accumulation of fat in ectopic depots, including the liver, visceral sites, myocardium, epi- and pericardial sites, perivascular sites and skeletal muscle (30). Excess lipid accumulation in liver and muscle cells directly impact on insulin sensitivity, resulting in increased glucose output in liver and decreased glucose uptake in muscle, which contributes to impaired glucose tolerance and type 2 diabetes (31). In adipocytes, impaired insulin sensitivity lead to breakdown of triglycerides (lipolysis) and increased release of free fatty acids and glycerol, as well as reduced glucose uptake (32). A recent study by Kim et al showed that hypertrophic adipocytes had a reduced ability for insulin-stimulated glucose uptake (33).This chain of reaction further enhances fat accumulation in liver and visceral depots, as well as development of insulin resistance. However, the

role of morphologic changes in SAT adipocytes are not straight forward. Recently, the "adipose tissue expandability"-hypothesis has been questioned and increase in small adipocytes have been linked to metabolic dysregulation, but this effect may be adipocyte site dependent (34).

A key feature of hypertrophic SAT expansion induces alterations in the secretion of pro-inflammatory cytokines, particularly IL-6, MCP-1 and G-CSF (4,35). A study by Heinonen et al. showed a correlation between increased adipocyte cell size and an increased expression of genes regulating cell death and inflammation in adipose tissue (5). The increased release of proinflammatory cytokines by adipocytes and recruited immune cells, especially macrophages, influence adipose cell function, stimulate lipolysis and impair insulin signaling. Macrophage infiltration is positively related to adipocyte size and since macrophages alter levels of insulin signaling molecules and GLUT4 expression in adipocytes they have an important role in development of adipose tissue insulin resistance. (4,36)

Heinonen et al. also showed a correlation between decreased expression of mitochondrial genes in hypertrophic SAT adipocytes and Camastra et al. recently demonstrated that expanded SAT adipocytes are associated with small mitochondria, thin cytoplasm and degenerative signs (4,5). These morphological changes in hypertrophic adipocytes make them more prone to rupture and cell death, with macrophage infiltration and further inflammation as a result. Expanded adipocytes are in fact associated with a higher density of crown-like structures, which are structures seen in adipose tissue formed by macrophages in the process of removing dead adipocytes. (4)

Adipose tissue expansion is also associated with lower adiponectin levels (4). Adiponectin is an adipocytokine secreted by adipose tissue and it exerts numerous metabolically favorable actions on several tissues. In adipose tissue, adiponectin is associated with healthy adipose tissue expansion and prevention of ectopic lipid accumulation. Studies have also shown that in near complete lack of insulin, adiponectin is critical for insulin signaling and lipid uptake in SAT. In liver, adiponectin decreases lipogenesis, enhances β-oxidation and inhibits hepatic gluconeogenesis, thereby

preventing intrahepatic lipid accumulation and decreasing glucose output. In skeletal muscle, adiponectin has been linked to increased levels of fatty acid oxidation, thus preventing intramuscular lipid accumulation and enhanced GLUT4-mediated glucose uptake. Pancreatic β-cells express receptors for adiponectin and recent studies have shown that adiponectin prevents the attenuation of glucose-stimulated insulin secretion in β-cells challenged with lipid overload. Additionally, reduced levels of adiponectin have been associated with cardiovascular disease, which may be explained by the ability of adiponectin to stimulate angiogenesis, inhibit apoptosis and promote nitric oxide synthesis in endothelial cells. (37) Low levels of adiponectin seen in obesity may be a result of a reduction in adiponectin gene expression caused by pro-inflammatory cytokines, such as TNF-α and IL-6. Hypertrophic adipocytes may therefore indirectly through increased release of pro-inflammatory cytokines and recruitment of immune cells reduce levels of circulating adiponectin and augment the risk for metabolic disease. (38,39)

The impact of fructose on the size and function of SAT adipocytes is largely unknown. Mature adipocytes mainly express GLUT4 transporters, which are highly selective to glucose, suggesting that fructose may not have a direct impact on SAT adipocytes. The crosstalk between liver and adipose tissue is important for regulation of whole-body energy homeostasis and impaired signaling between these tissues may explain the various adverse metabolic effects high fructose consumption can cause.

# 3. Materials and methods

### *3.1 Study cohort*

The study in question is a subgroup of two already published fructose intervention studies. (7,40). In brief, healthy men were recruited via newspaper advertisements at four centers: in Helsinki, Finland; Naples, Italy; Quebec, Canada; and Gothenburg, Sweden. This study includes only study subjects recruited in Helsinki from whom fat biopsies were taken both before and after fructose intervention. Thus, a total of 34 obese healthy men participated in the present study. Inclusion criteria were men aged

20-65 years with large waist circumference (>96 cm), body mass index (BMI) 27-40 kg/m, stable weight (±3 kg) for at least the last 3 months, LDL-cholesterol < 4.5 mmol/l and serum triglycerides < 5.5 mmol/l. Exclusion criteria were: smoking, alcohol consumption over 2 doses/day (i.e., 20 g pure alcohol), type 2 diabetes, cardiovascular disease, hormonal therapy, hepatic and renal diseases, gastroenterological, thyroid or hematological abnormalities, and any chronic disease requiring medication except for controlled hypertension.

### *3.2 Fructose intervention*

The subjects underwent a 12-week fructose intervention period, during which they consumed 75 g of fructose each day. Fructose was administered three times daily as a carbonated beverage prepared as 7,6% (w/w) solutions and flavored with lemon aroma (specially produced for this study by Nokian Panimo Oy, Finland). The lemonade was served in 330 ml bottles equaling 990 ml and 303 kcal per day. Subjects were instructed to consume the beverages together with the three main meals while following their habitual ad libitum diet and physical activity. The beverage was well tolerated. Each subject kept a food record for 3 days, 2 work days and one day off, before the fructose intervention and within 2 weeks after. A qualified nutritionist gave detailed verbal and written instructions for filling in the food record. The nutritionist also contacted the participants every 1-2 weeks to assure compliance with the fructose intervention.

### *3.3 Determination of adipocyte size*

For a detailed review over adipocyte size determination, please see reference 5. Briefly, biopsies of subcutaneous adipose tissue were obtained under local anesthesia from the abdominal area under the umbilicus by needle aspiration. The subcutaneous adipose tissue was minced and incubated for 1 hour at 37°C with continuous shaking in 10 ml of adipocyte medium (DMEM/F-12 (1:1) (Invitrogen, Paisley, UK) supplemented with 16 µmol $l^{-1}$ biotin, 18 µmol $l^{-1}$ panthotenate, 100 µmol $l^{-1}$ ascorbate and antibiotic-antimycotic (Invitrogen)), supplemented with 2 % bovine serum albumin (Sigma, St Louis, MO, USA) and with 2 mg $ml^{-1}$ collagenase A (Roche, Basil, Switzerland). Digestion was stopped when the adipocyte medium supplemented with 10 % newborn calf serum (Sigma) was added, and suspended cells were then centrifuged for 10 min at 600 *g*. After

washing the adipocytes with adipocyte medium, photographs of the adipocytes were then taken with a light microscope (Zeiss, Axioplan2) at magnification x50, using three different cameras at different phases corresponding to 2.12, 1.34 or 0.80 micrometers per pixel. The diameters of a maximal number of cells (cell count ranging from 140 to 2317) were measured using image processing and analysis software ImageJ (ImageJ 1.42 q / Java 1. 6.0_10 32-bit). Mean adipocyte diameter and volume were then calculated for each adipocyte, for formulas used see reference 5.

### *3.4 Determination of fat depots and biochemical analyses*

Please see reference 7 and 40 for a detailed review over the determination of liver, subcutaneous and visceral fat depots and biochemical analyses.

### *3.5 Statistical analyses and calculations*

IBM SPSS Statistics (Version 24, NY, USA) was used for statistical analyses. Data are presented as mean ± standard deviation and range. Nonparametric tests (the Wilcoxon Matched-Pair Signed-Rank test to continuous data when comparing two specified variables) were used for comparing data before and after fructose intervention. Independent sample T-test was used for comparing means between groups (Equality of variances were determined with Levene's test). Spearman's rank test was used for calculating correlations. *P* values < 0,05 were considered statistically significant.

## 4. Results

### *4.1 Basic characteristics*

Baseline data of the 34 men, aged 42–65 years, who completed the 12-week fructose intervention are shown in Table 1. All subjects were obese with mean BMI of 30,4 ± 2,9 kg $m^{-2}$.

At baseline, the subjects showed a wide range in BMI (26,5–36,2 kg $m^{-2}$), liver fat (0,5–19,4 %) and adipocyte diameter (61,6–123,1 µm). BMI correlated with waist circumference (r=0,76, P<0,0001), visceral (r=0,47, P=0,005) and subcutaneous fat (r=0,70, P<0,0001), but not with liver fat content. Visceral fat did not correlate with liver

| | **Before** | | **After** | | **Change** | |
|---|---|---|---|---|---|---|
| | Mean ± SD | Range | Mean ± SD | Range | Mean ± SD | P-value |
| Age (years) | 51,9 ± 7,1 | 42–65 | | | | |
| Waist (cm) | 109,3 ± 6,9 | 97,0–124,5 | | | | |
| Weight (kg) | 100,5 ± 10,8 | 73,1–118,7 | 101,0 ± 11,0 | 73,2–120,8 | 0,6 ± 2,5 | 0,163 |
| BMI (kg $m^{-2}$) | 30,4 ± 2,9 | 26,5–36,2 | 30,6 ± 3,0 | 26,2–36,0 | 0,18 ± 0,75 | 0,164 |
| Glucose (mmol $L^{-1}$) | 5,6 ± 0,4 | 4,9–6,2 | 5,6 ± 0,5 | 4,8–6,8 | 0,07 ± 0,46 | 0,325 |
| Insulin (pmol $L^{-1}$) | 87,2 ± 31,6* | 19–140 | 86,1 ± 38,1* | 29–173 | 0,6 ± 35,6 | 0,993 |
| Adiponectin (ng $ml^{-1}$) | 4,3 ± 3,1 | 1,3–12,3 | 4,2 ± 2,7 | 1,5–12,1 | -0,16 ± 1,00 | 0,893 |
| Tot-chol (mmol $L^{-1}$) | 5,1 ± 0,8 | 3,5–7,1 | 5,2 ± 0,9 | 3,4–7,8 | 0,06 ± 0,53 | 0,620 |
| HDL-chol (mmol $L^{-1}$) | 1,1 ± 0,3 | 0,5–2,0 | 1,1 ± 0,3 | 0,7–2,0 | -0,01 ± 0,15 | 0,592 |
| LDL-chol (mmol $L^{-1}$) | 3,5 ± 0,7* | 2,2–5,3 | 3,5 ± 0,8 | 2,2–5,5 | 0,06 ± 0,43 | 0,477 |
| FFA (µmol $L^{-1}$) | 422,6 ± 135,7 | 127–671 | 454,8 ± 127,3 | 173–838 | 32,2 ± 156,1 | 0,297 |
| Triglycerides (mmol $L^{-1}$) | 1,44 ± 0,66* | 0,67–3,35 | 1,60 ± 0,74 | 0,54–3,90 | 0,14 ± 0,40 | **0,034** |
| β-OH butyrate (mg $dL^{-1}$) | 0,81 ± 0,65 | 0,14–2,55 | 0,65 ± 0,65 | 0,11–3,67 | -0,16 ± 0,86 | 0,116 |
| Liver fat (%) | 5,5 ± 4,2 | 0,5–19,4 | 6,4 ± 4,8 | 0,6–20,4 | 0,84 ± 2,0 | **0,011** |
| Visceral fat ($cm^3$) | 2624 ± 906 | 1203–4944 | 2878 ± 877 | 1301–5397 | 53,6 ± 391,0 | 0,239 |
| Subcutaneous fat ($cm^3$) | 3842 ± 1237 | 1841–8491 | 3960 ± 1216 | 1832–8099 | 18,3 ± 257,0 | 0,570 |
| Adipocyte diameter (µm) | 104,7 ± 12,0 | 61,6–123,1 | 103,4 ± 13,0 | 71,8–126,6 | -1,27 ± 11,22 | 0,417 |

**Table 1** *Characteristics of the study subjects (n=34) before and after 12-week fructose intervention period. Adipocyte diameter was measured from 34 subjects. The data are mean ± SD. Ranges are indicated as minimum and maximum values. Changes of the means after versus before are shown with ± SD. P-values have been calculated using the Wilcoxon signed-rank test. Statistically significant changes (P-value < 0,05) are bolded.*

** N=33*

fat or subcutaneous fat and liver fat did not correlate with subcutaneous fat. Adiponectin levels correlated positively with plasma HDL-cholesterol (r=0,387, P=0,024), β-OH butyrate (r=0,536, P=0,001) and serum free fatty acids (r=0,412, P=0,015).

### *4.2 Determinants of adipocyte size at baseline*

Mean subcutaneous adipocyte diameter before fructose intervention was measured from 34 subjects and ranged from 61,6–123,1 µm. First, we studied the relationship between the adipocyte size and body fat depots. The adipocyte size did not correlate with BMI, subcutaneous fat, liver or visceral fat.

Secondly, we studied the relationship between the adipocyte size and blood lipid levels and metabolic parameters. The adipocyte size at baseline did not show any correlations with blood lipid levels, glucose, insulin or adiponectin.

### *4.3 Metabolic changes during fructose intervention*

Subject characteristics after 12-week fructose intervention period are also shown in Table 1. There was a significant increase in triglyceride levels (mean change 0,14 ± 0,40 mmol $L^{-1}$, P=0,034) and liver fat content (0,84 ± 2,0 %, P=0,011). Changes in liver fat correlated with changes in serum free fatty acids (r=0,363, P=0,035). Changes in weight correlated with changes in subcutaneous fat (r=0,441, P=0,009). Changes in adiponectin levels correlated with changes in total cholesterol (r=0,383, P=0,025) and β-OH butyrate levels (r=0,340, P=0,049).

Adipocyte diameter was measured from 34 subjects after fructose feeding. The mean change in adipocyte diameter was -1,27 ± 11,22 µm, which was not statistically significant (P=0,417). Changes in adipocyte size did not correlate with changes in weight, subcutaneous, visceral or liver fat. Changes in adipocyte size did not correlate with changes in blood lipid, glucose, insulin or adiponectin levels.

### *4.4 Subgroups with large and small adipocyte diameter*

There was a large variation in adipocyte diameter between subjects at baseline and thus we divided the subjects in to groups of two: subjects with small adipocytes with a diameter under the median value (< 105.95 µm, N=17) and subjects with large

adipocytes with a diameter over the median value (> 105.95 µm, N=17). Mean values of metabolic parameters in subjects with small and large adipocytes, respectively, are shown in Table 2. At baseline, there were no statistically significant differences between the two groups.

Next, we wanted to see if subjects with small and large adipocytes responded differently to fructose intervention. Metabolic changes between the two groups during fructose intervention are shown in Table 3. There were no statistically significant differences between the two groups after fructose intervention. However, there was a statistically significant increase in liver fat content (P=0,012) after fructose intervention in subjects with small adipocytes at baseline, which was not seen in subjects with large adipocytes at baseline. Subjects with large adipocytes at baseline, on the other hand, showed a significant increase in triglyceride levels (P=0,044) after fructose intervention, which was not seen in subjects with small adipocytes at baseline. Changes in triglyceride levels in subjects with large adipocytes at baseline correlated with changes in total cholesterol levels (r=0,596, P=0,015). Furthermore, subjects with large adipocytes at baseline showed a mean increase in insulin levels after fructose intervention, whereas subjects with small adipocytes at baseline showed a mean decrease in insulin levels after fructose intervention. The different response in insulin levels was, however, not statistically significant (P=0,054).

### *4.5 The impact of adipocyte size change during fructose intervention*

Taking the whole study group in to account there were some subjects who displayed reduction in the adipocyte diameter after fructose intervention, while others responded by an increase in the adipocyte diameter. Therefore, we next wanted to analyze whether there is a difference in metabolic changes between subjects with diverse response in adipocyte size after fructose intervention.

From the 34 study subjects, 18 subjects showed a negative change (i.e. reduction) in adipocyte size (mean change -9,86 ± 5,78 µm) and 16 subjects showed a positive change (i.e. increase) in adipocyte size (mean change 8,39 ± 7,2 µm) after fructose intervention.

| | **Small adipocytes** | **Large adipocytes** | **Difference** | |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean difference ± SD | P-value |
| Age (years) | 52,2 ± 7,8 | 51,7 ± 6,5 | 0,59 ± 2,5 | 0,813 |
| Weight (kg) | 99,7 ± 13,0 | 101,2 ± 8,4 | -1,42± 3,74 | 0,706 |
| BMI (kg $m^{-2}$) | 30,4 ± 3,0 | 30,4 ± 2,9 | 0,002 ± 1,000 | 0,999 |
| Waist (cm) | 110,2 ± 7,3 | 108,2 ± 6,1 | 1,71 ± 2,40 | 0,483 |
| Glucose (mmol $L^{-1}$) | 5,6 ± 0,4 | 5,5 ± 0,4 | 0,05 ± 0,13 | 0,697 |
| Insulin (pmol $L^{-1}$) | 89,8 ± 32,5 | 84,7 ± 31,5 | 5,17 ± 11,14 | 0,646 |
| Adiponectin | 4,3 ± 3,3 | 4,4 ± 3,0 | -0,17 ± 1,08 | 0,879 |
| Tot-chol (mmol $L^{-1}$) | 5,2 ± 0,8 | 5,0 ± 0,9 | 0,28 ± 0,28 | 0,329 |
| HDL-chol (mmol $L^{-1}$) | 1,2 ± 0,3 | 1,1 ± 0,3 | 0,09 ± 0,11 | 0,428 |
| LDL-chol (mmol $L^{-1}$) | 3,5 ± 0,7 | 3,4 ± 0,7 (n=16) | 0,13 ± 0,24 | 0,592 |
| FFA (µmol $L^{-1}$) | 427,4 ± 108,0 | 417,9 ± 162,0 | 9,47 ± 47,22 | 0,842 |
| Triglycerides (mmol L-1) | 1,50 ± 0,64 | 1,37 ± 0,70 | 0,13 ± 0,23 | 0,574 |
| β-OH butyrate (mg $dL^{-1}$) | 0,82 ± 0,69 | 0,81 ± 0,63 | 0,01 ± 0,23 | 0,959 |
| Liver fat (%) | 5,3 ± 3,4 | 5,8 ± 5,0 | -0,48 ± 1,46 | 0,744 |
| Visceral fat ($cm^3$) | 2718 ± 933 | 2930 ± 893 | -211 ± 313 | 0,505 |
| Subcutaneous fat ($cm^3$) | 4011 ± 1612 | 3872 ± 742 | 140 ± 430* | 0,748 |
| Adipocyte diameter (µm) | 96,9 ± 11,8 | 112,4 ± 5,2 | -15,53 ± 3,13 | **< 0,0001** |

**Table 2**. *Baseline characteristics of subjects with small (N=17) and large (N=17) adipocytes as well as their mean differences. Data is shown as mean ± SD. Statistically significant differences (P-value < 0.05) are bolded.*

**Equal variances not assumed (Levene's test).*

| **Metabolic changes in subjects with:** | **Small adipocytes** | **Large adipocytes** | **Difference** | |
|---|---|---|---|---|
| | Mean change ± SD | Mean change ± SD | Mean ± SD | P-value |
| Weight (kg) | 0,34 ± 2,20 | 0,85 ± 2,79 | -0,51 ± 0,86 | 0,556 |
| BMI (kg $m^{-2}$) | 0,11 ± 0,67 | 0,25 ± 0,84 | -0,14 ± 0,26 | 0,596 |
| Glucose (mmol $L^{-1}$) | -0,08 ± 0,38 | 0,21 ± 0,50 | -0,29 ± 0,15 | 0,067 |
| Insulin (pmol $L^{-1}$) | -12,20 ± 30,67 | 11,88 ± 36,59 | -24,08 ± 12,03 | 0,054 |
| Adiponectin (ng $ml^{-1}$) | -0,36 ± 1,23 | 0,05 ± 0,69 | -0,41 ± 0,34 | 0,238 |
| Tot-chol (mmol $L^{-1}$) | 0,009 ± 0,52 | 0,11 ± 0,55 | -0,10 ± 0,18 | 0,586 |
| HDL-chol (mmol $L^{-1}$) | 0,007 ± 0,17 | -0,04 ± 0,13 | 0,043 ± 0,052 | 0,412 |
| LDL-chol (mmol $L^{-1}$) | 0,006 ± 0,43 | 0,12 ± 0,44 | -0,11 ± 0,15 | 0,464 |
| FFA (µmol $L^{-1}$) | 32,00 ± 120,61 | 32,29 ± 189,03 | -0,29 ± 54,38 | 0,996 |
| Triglycerides (mmol L-1) | 0,06 ± 0,41 | **0,22 ± 0,38*** | -0,17 ± 0,14 | 0,238 |
| β-OH butyrate (mg $dL^{-1}$) | -0,29 ± 0,66 | -0,04 ± 1,04 | -0,25 ± 0,30 | 0,403 |
| Liver fat (%) | **1,20 ± 1,63*** | 0,49 ± 2,23 | 0,70 ± 0,70 | 0,304 |
| Visceral fat ($cm^3$) | -23,41 ± 454,90 | 130,59 ± 310,04 | -154,00 ± 133,52 | 0,257 |
| Subcutaneous fat ($cm^3$) | 20,71 ± 288,29 | 15,94 ± 229,88 | 4,77 ± 89,43 | 0,958 |
| Adipocyte diameter (µm) | -0,44 ± 13,05 | -2,10 ± 9,37 | 1,65 ± 3,90 | 0,674 |

**Table 3.** *Metabolic changes in subjects with small and large adipocytes and their mean differences. Data is shown as mean ± SD. Statistically significant differences (P-value < 0.05) are bolded. Significant within group changes are indicated by *. P-values are between-group differences.*

At baseline the two groups showed significant differences in weight, β-OH butyrate and subcutaneous fat. Increase in adipocyte diameter was associated with lower weight (mean difference 7,25 ± 3,47 kg, P=0,045), more subcutaneous fat (mean difference 881,52 ± 402,66 $cm^3$, P=0,036) and higher β-OH butyrate levels (mean difference 0,44 ± 0,21 mg $dL^{-1}$, P=0,049) at baseline. Subjects with an increase in adipocyte diameter also had a smaller mean adipocyte diameter at baseline than subjects with a reduction in adipocyte diameter. Mean adipocyte diameter at baseline in the group of subjects with an increase versus decrease in adipocyte diameter after fructose intervention was 100,5 µm and 108,3 µm, respectively. Yet, the difference in mean adipocyte size at baseline between the groups was almost but not entirely statistically significant (P=0,056).

Subjects who showed a reduction in adipocyte diameter showed a significant increase in liver fat content (mean change 1,16 ± 1,97 %, P=0,023) after fructose intervention. The reduction in adipocyte size correlated with a decrease in triglyceride levels (r=0,486, P=0,048). The increase in liver fat content correlated with an increase in glucose levels after fructose intervention (r=0,484, P=0,042). Subjects who showed an increase in adipocyte diameter after fructose intervention did not show a significant increase in liver fat content (mean change 0,49 ± 1,94 %, P=0,214).

### *4.6 The impact of liver fat change during fructose intervention*

Additionally, some subjects showed increased liver fat content after fructose intervention while liver fat was reduced among others. To further analyze differences between these opposite responses to fructose intervention in liver fat content, we divided the subjects in to two groups according to their loss or gain of liver fat.

9 subjects had a reduced liver fat content (from 5,6 ± 3,6 to 4,2 ± 3,6 %). There was no significant change in adipocyte size and changes in adipocyte size did not correlate with reduced liver fat content. This group showed a mean decrease in subcutaneous fat (-85,33 ± 160,88), however not statistically significant. Changes in adipocyte size in this group correlated positively with changes in glucose levels (r=0,709, P=0,032).

Increase in liver fat content was seen in 23 subjects (from 5,3 ± 4,5 to 7,1 ± 5,1 %). This group also showed a mean increase in subcutaneous fat (mean change was 81,00 ± 264,6

$cm^3$), which was not statistically significant. However, the different response in subcutaneous fat content after fructose intervention between the groups with either a gain or loss in liver fat was statistically significant (mean difference 193,7 ± 89,2 $cm^{3,}$ P=0,037). The change in subcutaneous fat in this group correlated positively with changes in adipocyte size (r=0,444, P=0,034). In this group there was not a correlation between changes in adipocyte size and glucose levels, as were seen in the group with reduced liver fat content. Changes in adiponectin levels after fructose intervention in this group correlated with changes in total plasma cholesterol (r=0,532, P=0,009), but not with either plasma LDL-cholesterol or plasma HDL-cholesterol. 2 subjects showed no change in liver fat content after fructose intervention.

# 5. Discussion

In the present study we investigated if mean SAT adipocyte size is a determinant of metabolic health or determines the effect of 12-week fructose consumption (75 g/day) on liver fat, DNL, fat oxidation, lipid metabolism or insulin sensitivity among obese men with features of the metabolic syndrome.

At baseline, the mean SAT adipocyte size did not correlate with size of fat depots, blood lipid levels, glucose, insulin or adiponectin levels. Klöting et al. demonstrated that larger subcutaneous and omental adipocytes were associated with higher levels of triglycerides and free fatty acids and Camastra et al. showed that larger adipocytes were associated with lower adiponectin levels (4,29). Most probably this discrepancy in our results to previous studies is explained by our homogenous group consisting only from obese men all showing some features of the metabolic syndrome.

Adiponectin was as expected an important regulator of liver metabolism. At baseline, adiponectin correlated positively with plasma HDL-cholesterol, β-OH butyrate and serum free fatty acids. This supports previous findings of the numerous positive metabolic effects

adiponectin gives rise to in the liver and its important role in sustaining a healthy metabolic profile (37).

One of our main findings in the present study is that 12-week fructose intervention does not affect the SAT adipocyte size in subjects with obesity. Overall, fructose consumption had only minor effects on adipose tissue. Adipocytes express mainly the GLUT4 transporter, which is highly selective to glucose, and this might explain why fructose does not have a direct effect on adipose tissue. Fructose consumption did lead to a significant increase in liver fat content and triglyceride levels, consistent with previous studies (7,10). This is mainly due to the ability of fructose to promote hepatic DNL and release of triglyceride-rich VLDL particles, as reported in a recent study by Taskinen et al (7).

There was a large variation in adipocyte diameter between subjects at baseline and thus we divided the subjects in to groups of two according to adipocyte size median value: subjects with small adipocytes with a diameter under the median value (< 105.95 µm) and subjects with large adipocytes with a diameter over the median value (> 105.95 µm). At baseline, subjects with large and small adipocytes did not differ significantly in any other measured metabolic parameter, therefore following findings are likely to be explained by adipocyte size alone.

We found that subjects with small adipocytes at baseline showed a significant increase in liver fat content after fructose intervention. Increase in liver fat content was not seen in subjects with large adipocytes at baseline. As discussed above, our results and previous studies have shown that fructose consumption induces fat accumulation in liver. Interestingly, our findings suggest that small SAT adipocytes are associated with greater gain in liver fat in response to fructose consumption. The current concept of limited SAT expandability theory state that when SAT adipocytes reach their maximal expansion capacity they will not be able to further store lipids, which lead to a spillover of lipids that consequently accumulate in ectopic depots (30). Perhaps, small adipocytes reach their maximal capacity to expand at an earlier stage and therefore a higher amount of lipids accumulates in the liver.

On the other hand, subjects with large adipocytes at baseline showed a significant increase in triglyceride levels after fructose intervention. The increase in triglyceride levels in this group correlated positively with changes in total cholesterol levels. Thus, subjects with large SAT adipocytes at baseline showed a worsened and more atherogenic lipid profile after fructose intervention. These findings could also be explained by the limited SAT expandability theory (30). In the case of large adipocytes, the spillover of lipids is seen as increased triglycerides in the circulating blood.

Furthermore, subjects with large adipocytes at baseline showed a mean increase in insulin levels after fructose intervention, compared to subjects with small adipocytes at baseline who showed a mean decrease in insulin levels after fructose intervention. The different response in insulin levels was borderline statistically significant. Still, it could suggest a decreased insulin sensitivity in subjects with large adipocytes. We speculate if this might be due to macrophages infiltrating the hypertrophic adipose tissue and altering insulin signaling pathways and GLUT4 expression in adipocytes. Previous studies have linked larger adipocytes to inflammatory conditions and adipose tissue macrophage infiltration (4,36). Moreover, the response in insulin levels seen in subjects with large adipocytes at baseline is not in line with a previous study by Johannsen et al., which found that small adipocytes responded to 8-week overfeeding with decreased insulin sensitivity (34). Speculatively, the different result is a consequence of a fructose-specific diet.

Finally, we found that some subjects displayed a reduction in SAT adipocyte diameter after fructose intervention, while others responded by an increase in adipocyte size. Subjects who showed a reduction in adipocyte diameter showed a significant gain in liver fat after fructose intervention, whereas subjects with increased adipocyte diameter after fructose intervention did not display a gain in liver fat. The inability of the SAT adipocytes to expand seem to lead to increased uptake of lipids by the liver, again, in line with the limited SAT expandability theory (30). We could also see that subjects who increased their SAT adipocyte size after fructose intervention had smaller adipocytes at baseline, whereas subjects who reduced their SAT adipocyte size had larger adipocytes at baseline. Although, this result was not statistically significant, it gives some additional support to the limited SAT expandability theory that large adipocytes cannot further expand and thus reduces

their adipocyte size with a subsequent fat accumulation in liver. Lastly, the gain in liver fat in subjects with reduced adipocyte size correlated with an increase in glucose levels, suggesting decreased insulin sensitivity in these subjects. Thus, a reduction in adipocyte size is associated with a more diabetogenic profile.

In summary, SAT adipocyte size was not a major determinant of metabolic health in obese men with features of the metabolic syndrome and added dietary fructose 75 g per day for 12 weeks did not result in changes in adipocyte size. In the present study we could show that subgroup of subjects with small SAT adipocytes or subjects who show a reduction in their SAT adipocyte size display greater fat accumulation in the liver during fructose intervention which is compatible with adipocyte expandability hypothesis. The subgroup with large adipocytes, on the other hand, demonstrated worsened blood lipid profile but no liver fat gain. SAT adipocyte size and changes in size seem to be linked to multiple adverse metabolic changes during high fructose consumption in some but not all subjects with metabolic syndrome.

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